the interior of North America has not been invaded by marine waters.

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The Effects of X-Rays on the Mitotic Activity of Mouse Epidermis

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With the increased interest in various types of ionizing radiation as a result of the Atomic Energy Program, there is a great need for a practical method for the quantitative evaluation of the effects of sublethal doses of such radiation. Many investigators have shown that small doses of radiation result in a temporary but marked depression of the mitotic activity of lower animal, plant, embryonal, and tumor cells. This suggests that similar studies of mitosis in mammalian tissues might lead to a relatively simple and reasonably specific method of expressing radiation damage. The usual technique of determining the mitotic index of a tissue involves the actual counting of many thousands of individual cells. Since this is extremely laborious, considerable effort has been devoted to developing simpler, more expeditious methods. This preliminary report describes a simplified technique of obtaining the mitotic index of mouse skin and indicates the surprising sensitivity of the mitotic activity of mouse epithelium to the effects of X-rays.

Groups of animals (CF_1 strain white mice, 6–8 weeks of age) were exposed to specific doses of 250-KV peak voltage X-rays at the rate of 50 r/min and then autopsied at definite time intervals after exposure. Immediately after the animal had been killed by crushing the cervical spine, the ears were removed and placed in 1% acetic acid. After 16 hrs at 5° C, a homogeneous layer of epidermis two cells thick was separated from the dermis according to the technique mentioned by Hoepke (*3*) and described in detail by Cowdry (*2*). The section of epidermis was then stained with Mayer's hematoxylin and mounted on slides for study. The cells in mitosis (arbitrarily defined as the period between the breakdown of the nuclear membrane in prophase and the complete separation of the cytoplasm in telophase) in a given number of microscopic fields outlined by a Whipple disc were then counted. The number of epidermal cells in the field delimited by each Whipple disc was carefully determined for animals of the strain and age used in this study so that the final value of the mitotic index can be expressed in terms of mitoses/100,000 cells. Variation in cell numbers from field to field is statistical in nature and introduces an error of 1–2% not encountered when individual cells are counted. The much larger number of cells which can be examined practically by the field method compensates for this error by reducing the over-all statistical error. It has been shown that X-ray dosage up to 325 r does not significantly alter the number of cells per field, so this method is valid for mouse skin after radiation exposure.

The change in mitotic index of mouse epithelium produced over a range of sublethal doses of X-rays from 5 to 325 r has been studied. The graphic response of the mitotic index in animals receiving 35 and 325 r is shown in Fig. 1. Each point on the experimental curve represents the average mitotic index obtained by examining a total of approximately 200,000 epithelial cells in 4–5 experimental animals. The diurnal variation in mitotic activity has been taken into account in the exposure groups, since there is twice as much mitotic activity during the morning as there is in the evening. This has been previously reported (*1*) and confirmed in our laboratory by means of the control animals for the above experiments.

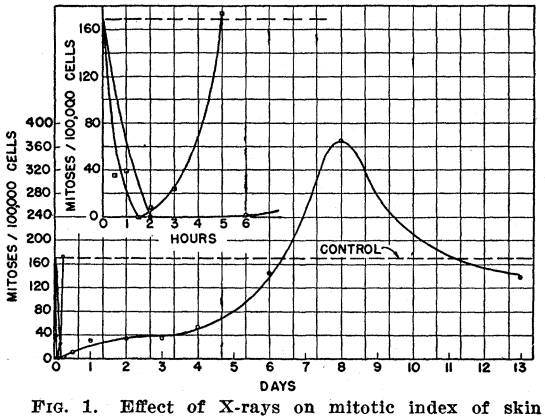

Fig. 1. Effect of X-rays on mitotic index of skin of the mouse: Broken line—Average control counts (average of 44 mice, 169/100,000 cells); Squares—35-r X-ray (5 mice/point); Circles—325-r X-ray (4 mice/point).

In both of the experimental groups the minimum point of mitotic activity is less than 1 mitosis/100,000 cells. This minimum was reached in less than 2 hrs after exposure. On the other hand, the time required for the mitotic index to return to normal varies from 5 hrs at 35 r to 6 days at 325 r. An "overcompensation" phenomenon is quite evident at the 325-r dosage level, with the mitotic activity more than double that of normal on the 8th day

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after irradiation. This phenomenon is being studied at the 35-r dosage level.

From the data presented above it is evident that the mitotic activity of mouse skin is extraordinarily sensitive to the effects of X-rays. Between the two dosages reported here it appears that the best index of damage is the time for the mitotic index to return to normal. Both the extent of the drop from normal and possibly the time in reaching the minimum point appear to be quite similar at these two extremes of dosage. However, the first point obtained at 325 r was at 2 hrs, and therefore the minimum point could have been reached earlier. By the use of this biological criterion of radiation effect our present program is to compare the relative destructiveness of different types and different energy-ionizing radiations.

It seems possible to postulate from the data at the dosage level of 35 r that the degree of depression of mitotic activity from normal may serve as an index of tissue damage at very low dosages. Experiments now in progress indicate that 5 r of 250-KV X-rays decreases mitotic activity to less than 25% of normal in 60–90 min.

The above work on the mitotic index in skin is being paralleled by similar studies in the jejunum, adrenals, and lymph nodes, but at the present time it appears that the skin is by far the most sensitive of the organs studied.

Experiments are in progress to determine the effect of rate of irradiation and of single or divided doses for various types of ionizing radiation on the mitotic index of mouse skin and other tissues. It is hoped that comparisons of the change in mitotic index and the shape of the recovery curve will be of value in evaluating these radiation effects.

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A Report on the Ridgway Color Standards

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Research workers have known for some time that the Ridgway Color Standards (*8*) are less useful in color description than it was originally hoped. Changes in hue, value, and chroma of the chips have resulted from aging, fading through exposure to strong light, offsetting, abrasion, and darkening through use. Moreover, there is no satisfactory way to describe those colors which occupy positions in the color space between named chips, since the spacing between the steps is quite variable. Since color records are still being made and reported in Ridgway terms, the authors believe that those biologists who are in the habit of using Ridgway, especially entomologists, mycologists, and ornithologists, would be interested in the visual differences noted among several Ridgway chips bearing the same color name.

The discrepancies were noted recently when checking the Munsell (*4*) notations for 96 colors from a set of Ridgway color chips used in Ottawa. As a result, this set was brought to Toronto and compared with two copies of Ridgway here. Notations of 12 colors were made in Baltimore from another copy. These notations are shown in Table 1. The Munsell Standards (*4*) were employed since they are convenient to use, and the work of Newhall (*5*), Nickerson (*6, 7*), and many others has demonstrated their stability, utility, and accuracy of notation. Further, the Munsell description system, like that of a recent edition of Ostwald (*1*), has the advantage of being permanently described in terms of the I. C. I. system (*2, 7*), which is internationally known and understood.

In considering the notations, some latitude must be given to inherent errors, errors of human judgment, errors produced by imperfections in viewing conditions and illumination, and errors which may possibly have arisen through the use of two sets of Munsell Standards, one in Toronto and the other in Baltimore. It is believed that the maximum error of figures shown in the table is within the limits of ± 0.5 hue, ± 0.25 value, and ± 0.5 chroma. Though the application of these limits to the recorded notations reduces the apparent differences in some cases, it should be kept in mind that the chips for which unlike notations are given were visually different when compared directly with one another.

The copies of Ridgway checked were: two copies from the Department of Botany, University of Toronto, one (Ta) purchased in 1929 and used steadily since then, one (Tb) purchased in 1940 and used rarely and only with great care; one copy (MB) from the Munsell Color Company, Baltimore; one copy (O) from the Department of Botany, Central Experimental Farm, Ottawa, purchased in 1919 and used since then. All copies have received careful treatment and have normally been stored in the dark. All were compared with a 40-hue set of Munsell Standards with occasional reference to the constant value and chroma sheets. Part of the Munsell Standards was purchased in 1940, the remainder in 1947.

During our notation both standard and unknown chips were masked with neutral gray, value 5, illuminated at 45° by either a Spencer Daylight lamp or north skylight, and viewed normally. Both types of lighting gave comparable results except in the cases of Vinaceous Cinnamon and Vinaceous Fawn. The skylight reading is used in both. In Baltimore a 6,500° K daylight lamp was used.

The data obtained are shown in Table 1. The first column gives only the colors for which the Munsell Color Company, Baltimore, provided a notation from its copy of Ridgway; the second column, the ISCC-NBS (Inter-Society Color Council—National Bureau of Standards) (*3*) class name as derived from the Munsell notation; the third, the copy index; the fourth, the Munsell notation; the fifth, the maximum differences in terms of hue, value, and chroma steps from the Tb copy of Ridgway. The ISCC-NBS class name was added, as it describes in simple terms the colors of the Ridgway chips.

During the comparison it was noted that in most cases the differences between the Tb copy, which was in very good condition, and the others were very easily seen, even